In vitro antibacterial and early stage biofilm inhibitory potential of an edible chitosan and its phenolic conjugates against *Pseudomonas aeruginosa* and *Listeria monocytogenes*

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Abstract

In the present study, the antibacterial potential of chitosan grafted with phenolics (CPCs) such as caffeic acid (CCA), ferulic (CFA), and sinapic acid (CSA) were evaluated against foodborne pathogens like *Pseudomonas aeruginosa* (PA) and *Listeria monocytogenes* (LM). The geometric means of minimum inhibitory concentration (MIC range 0.05–0.33 mg/ml), bactericidal concentration (MBC range 0.30–0.45 mg/ml), biofilm inhibitory concentration (BIC range 0.42–0.83 mg/ml), and biofilm eradication concentration (BEC range 1.71–3.70 mg/ml) of CPCs were found to be lower than the MIC (0.12–1.08 mg/ml), MBC (0.17–1.84 mg/ml), BIC (4.0–4.50 mg/ml), and BEC (17.4–23.0 mg/ml) of unmodified chitosan against PA and LM. CPCs attenuated the biofilms of PA and LM by increasing the membrane permeability of bacteria embedded within the biofilms. Further, sub MIC of CPCs ($0.5 \times \text{MIC}$) significantly reduced the biofilm adhesion ($p < 0.001$) by representative strains of LM (CCA: 72.2 ± 3.5 , CFA: 79.3 ± 0.9 , and CSA: $74.9 \pm 1.5\%$) and PA (CCA: 64 ± 1.1 , CFA: 67.8 ± 0.8 , and CSA: $65.7 \pm 4.9\%$). These results suggested the antibacterial and anti-biofilm potential of CPCs that can be exploited to control foodborne pathogenic infections.

Keywords Antibiofilm activity · Biofilm-forming bacteria · Chitosan-phenolic conjugates · Foodborne pathogenic bacteria

Introduction

Microbial contamination of the food materials and food contacting surfaces are the major cause for foodborne illness. *Listeria monocytogenes* (LM) is an important foodborne bacterium responsible for invasive infections such as septicemia,

meningitis, and listeriosis, a serious foodborne illness in immunocompromised, pregnant women, new borne, elderly people with high hospitalization and fatality rates (Gandhi and Chikindas 2007; Schuschat et al. 1991). *Pseudomonas aeruginosa* (PA) is an opportunistic pathogen found on food contacting surfaces and capable of causing sporadic infections in human (Rybtke et al. 2015). Further, as one of the member of ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella Pneumoniae*, *Acinetobacter baumannii*, and enterobacter spp), PA was reported to exhibit multiple drug resistance against wide range of antibiotics (Xie et al. 2017). The virulence trait of LM and PA to form biofilms on biotic (poultry, sea food, and vegetables) and abiotic (food packaging materials) surfaces allow these bacteria to survive prolonged periods under harsh environmental conditions (UV, desiccation, temperature and pH), resist biocide action (disinfectants, food preservatives, and decontaminants), and becomes frequent contaminants in food processing and packaging environments, especially minimally processed foods (Moltz and Martin 2005; Magalhaes and Nitschke 2013; Miao et al. 2017). Earlier, the pathogenic feature and characteristics of foodborne pathogen biofilms have been reported (Lin et al.

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2017). Due to recent trends in the emergence of biocide resistant pathogens in food chain (Condell et al. 2012; Capita and Alonso-Calleja 2013) and concerns regarding health safety of chemical preservatives (Kaplan 2010), studies have focused on the application of food compatible products to control LA and PA in ready to eat foods.

Chitosan (β -(1,4)-2-amino-2-deoxy-D-glucose) is a non-toxic biopolymer widely used in bio-medical, food, and agricultural industries (Cheung et al. 2015; Rinaudo 2006; No et al. 2007). Chitosan exhibits intrinsic antimicrobial activity against broad range of pathogens and this property has been used in edible coatings to extend the shelf life of various foods materials like fruits, vegetable, meat, poultry, and fermented foods (Wang et al. 2018). However, due to chitosan's poor water solubility, astringency, sensitivity to humidity, and interaction with food matrices, using various enzymatic and nonenzymatic methods, novel chitosan hybrid materials consisting of essential oils, antibiotics, metal complexes, and polysaccharides have been reported (Chen et al. 2009; Zhang et al. 2013, 2018; Patale and Patravale 2011) with improved antimicrobial, antioxidant, and water solubility properties. Nevertheless, their practical applications in the fields of food are rarely reported.

Hydroxycinnamic acids (HCAs) like caffeic, ferulic, and cinnamic acids are food components with antioxidant, anti-inflammatory, and hypolipidemic properties and widely used in food industries (Ghsarras 2009; Yang et al. 2016b). Phenolic acids grafted chitosan (CPCs) has been produced by various methods and the produced copolymers possessed improved antioxidant and antimicrobial properties (Liu et al. 2017). Yang et al. (2016a, b) produced various cinnamic acid derivatives of chitosan (caffeic and gallic acids) active against *Ralstonia solanacearum* and improved shelf life of mulberry fruits during storage. CPCs have been reported to kill planktonic cells of several foodborne pathogens and improve antioxidant and emulsifying ability (Lee et al. 2014; Lee and Je 2013; Lei et al. 2013). In addition to these reports, earlier we reported about the antibacterial effects of ferulic acid grafted chitosan against methicillin-resistant *S. aureus* (Eom et al. 2016). Although CPCs have been reported to exhibit antibacterial property, to the best of our knowledge, the anti-biofilm potential of CPCs has not been investigated against PA and LM. Therefore, in the present study we investigated the antimicrobial, anti-biofilm potential of CPCs against PA and LM.

Materials and methods

Chemicals and reagents

Chitosan (MW, 310 kDa) was purchased from Kitto life Co. (CAS-COS-KL225, Seoul, Korea).

4',6-diamidino-2-phenylindole (DAPI), Propidium Iodide (PI), phenolics (caffeic, ferulic, and sinapic acid), and phosphate buffered saline (PBS, pH 7.4) were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Preparation of CPCs

The CPCs were prepared according to the method (Cho et al. 2011) with slight modification. Briefly, the chitosan (0.5 g) was dissolved in 50 ml of 1% acetic acid (v/v) and then 1 ml of hydrogen peroxide (1.0 M) containing 0.054 g of ascorbic acid was added. After 30 min, phenolic acids (0.25 g) were added to the mixture and allowed to incubate at 25 °C for 24 h. The mixture was dialyzed against distilled water for 48 h, and the retentate was freeze dried. The identity of the products was confirmed by comparing with the ¹H NMR spectral data of authentic CPCs earlier reported (Lee et al. 2014).

Cultures and growth conditions

Pseudomonas aeruginosa KCCM 11321 was obtained from the Korean Culture Center of Microorganisms (KCCM; Seoul, Korea). *Listeria monocytogenes* standard strain KCTC 3569 was purchased from the Korean Collection for Type Cultures (KCTC; Daejeon, Korea). Clinical strains of *P. aeruginosa* (48, 152, 366, 1842, 2179, and 3248) and *L. monocytogenes* (2148, 2637, and 2868) were obtained from the Gyeongsang National University Hospital. The cultures were aerobically grown in tryptic soy broth (Difco Laboratory Inc., Detroit, MI, USA) supplemented with 1% glucose (TSBg) for 24 h at 37 °C prior to assays.

Effect of CPCs on planktonic cells

The minimum inhibitory concentration (MIC) of CPCs was determined by broth microdilution method (NCCLS 2003). The bacterial growth was determined by measuring the culture optical density at 610 nm (OD₆₁₀ nm) of the culture suspension at 610 nm using spectrophotometer (Multiskan GO, Thermo Fisher Scientific Korea Ltd., Seoul, Korea). The MIC is defined as the lowest concentration of CPCs that inhibited > 90% of the bacterial growth. Additionally, a 50 µl culture broth from the wells of micro titer plate containing 0.12, 0.25, 0.5, 1, and 2 × MIC of CPCs was aseptically withdrawn and spread on TS agar plate and further incubated till 48 h and the number of colonies formed on each plate was counted. The lowest concentration of CPC which resulted complete inhibition (minimum detection limit: < 5 colonies/plate) was considered as minimal bactericidal concentration (MBC). Similarly after 48 h, the highest concentration of CPCs which had no growth inhibitory effect against PA, LM was determined as sub MIC.

Effect of CPCs on preformed biofilms

The cultures of LM and PA were grown as mature biofilms by adding 0.2 ml of culture suspension (10^8 CFU/ml) in TSBg to the 96 well polystyrene plates. After 24 h incubation at 35 °C, the planktonic cells were gently removed and the wells were rinsed with PBS twice and drained. The biofilm-containing wells were filled with 0.2 ml of TSBg containing twofold serial dilutions of CPCs. The plates were further incubated at 35 °C for 24 h. The culture medium was transferred to clean microtiter plate and the OD₄₉₅ nm of the culture suspension at time 0 h and after incubation for 24 h was determined. The biofilm inhibitory concentration (BIC) was defined as the lowest concentration of CPCs which caused no growth in the supernatant fluid confirmed by no increase in OD₄₉₅ of supernatant compared to initial readings (Nostro et al. 2007). For biofilm eradication assay, after removal of planktonic cells from microtiter plate, PBS (0.2 ml) was added to each well and the plate was briefly sonicated and each well was scarified with sterile loop. The detached biofilm in PBS (0.2 ml) was then spread over the surface of TSA plate and incubated for 72 h at 37 °C. The lowest concentration of CPCs which prevented the growth of biofilm bacterial colonies on the agar surface was determined as minimum biofilm eradication concentration (MBEC).

Fluorescence microscopy

Mature biofilms (24 h) of PA and LM were grown on polystyrene cover slips submerged in 1 ml of TSBg containing $0.5 \times$ MBEC of CPCs (CCA, CFA at 2.04 mg/ml, CSA at 1.02 mg/ml for PA; all CPCs at 2.04 mg/ml against LM) in 24-well plates for 24 h. After the media was aspirated, the biofilm containing coverslip was transferred to a new well and then filled with PBS twice to ensure adequate removal of non-adherent cells. The coverslip with firm biofilm was then fixed with 4% paraformaldehyde for 30 min at 28 °C, residual fixative was removed, and the fixed biofilms were incubated with 1 ml PBS containing DAPI (2 µM) or PI (5 µM) for 5 min each at 28 °C. Four random areas for each sample (run in triplicate) were focused with 10× objective lens and images were acquired using confocal laser scanning microscope (CKX53, Olympus Inc., Tokyo, Japan) using λ_{ex} 314 nm and λ_{em} 410 nm (DAPI) and λ_{ex} 510 and λ_{em} 590 (PI).

Effect of CPCs on biofilm formation

The biofilm formation was quantified according the method previously described (Nostro et al. 2007) with some modifications. Briefly, 96 well microtiter plates containing 0.1 ml of sub MIC of CPCs (0.5, 0.25, and $0.12 \times$ MIC) prepared

in TSBg was inoculated with 0.1 ml of culture suspension (5×10^8 CFU/ml) in TSBg and incubated for 24 h at 37 °C. The planktonic cells containing media was aspirated and the wells were replaced with 0.2 ml of sterile PBS twice and discarded and the plates were dried at 38 °C for 30 min. The biofilm in the wells were stained with 0.4% safranin for 1 min followed by washing with PBS twice. The stained biofilm was extracted with 30% acetic acid (0.2 ml) and the absorbance of the supernatant was read at 475 nm using microplate reader (GENios® microplate reader; Tecan Austria GmbH; Grödig, Austria). As a measure of efficacy, the relative biofilm formation was defined as follows: (mean OD₄₉₂ treated well)/(mean OD₄₉₂ control well) $\times$ 100.

Statistical analysis

Each experiment was repeated twice with triplicates ($n=6$). The data representation, the geometric mean (G -mean) values which are transformed data derived using logarithms to generate a normal distribution was used (Bland and Altman 1996). G -mean values were compared using two tailed t tests using the Graph Pad Quick calcs. (<http://www.graphpad.com/quickcalcs/ttest1.cfm>).

Results and discussion

Characterization of CPCs

The ^1H NMR spectra of unmodified chitosan and CPCs were presented in Fig. S1. The UMC showed peaks at 2.0 ppm (methyl protons of acetylated glucosamine), 3.41 and 4.30 ppm (protons of C-3, C-4, C-5 and C-6 of the pyranose ring), 3.14 and 4.83 ppm (protons of C-2 and C-5 of the glucosamine residues). In case of CPCs, new peaks 6.32 and 8.22 (protons of C-2, C-5, C-6, C-7, and C-8 of phenolic acids). These results indicated that phenolics were successfully grafted to chitosan and the products were designated as chitosan–caffeic acid (CCA), -ferulic acid (CFA) and -sinapic acid (CSA), respectively. The grafting efficacy of CPCs is given in Table S2.

Antibacterial activities of CPCs against planktonic cells

In the present study the antimicrobial activity of CPCs against planktonic cells of LM and PA was evaluated. From Table 1 and S1, it is evident that UMC exhibited 1.7 and 1.4 fold increase in the mean MBC value over its MIC against PA (MIC range 1.02–4.04 mg/ml) and LM strains (MIC range 0.06–0.51 mg/ml) and these values are comparable with the MIC and MBC reported for chitosan against PA (3 mg/ml) and LM (0.15–0.8 mg/ml) strains (Tao et al. 2011;

Goy et al. 2009). However, CPCs exhibited better antimicrobial effect (MIC *G*-mean range, 0.22–0.33 mg/ml for PA and 0.03–0.45 mg/ml for LM) and these values are comparable with the MIC of CFA, CCA and CSA against PA (range, 0.25–0.51 mg/ml) and LM (0.03–0.06 mg/ml) planktonic cells (Lee et al. 2014). Compared to our results, Chatterjee et al. (2015) reported higher MIC for gallic, ferulic, coumaric, and valine grafted chitosan (range 0.62–5 mg/ml against PA and 0.43–0.87 mg/ml against *S. aureus*) probably due to low MW of chitosan (100 kDa) and the choice of media (nutrient broth) used for MIC assays. Further, high MW chitosan compared to low MW was better antimicrobial against Gram-positive bacteria (Zheng and Zhu 2003). The MBC observed for CPCs (*G*-mean range, 0.30–0.45 mg/ml for PA and 0.06–0.12 mg/ml for LM) is comparable to the MBC of catechin loaded chitosan (0.062–0.12 mg/ml) against LM and *E. coli* (Zhang et al. 2016). Compared to the MBC values reported by Borges et al. (2012) for ferulic, gallic acids (0.5 mg/ml against PA and 5 mg/ml against LM), the CPCs contained significantly lower amount of phenolics (Table S2).

The observation that PA is less susceptible to CPCs than LM is in line with the observations of Božič et al. (2012) who reported that gallic acid grafted chitosan exhibited higher MIC and MBC against Gram-negative bacteria. This differential effect of CPCs against PA and LM may be due to the differences in the cell wall composition and the membrane architecture. Chitosan, gallic acid grafted chitosan were shown to interact with lipoteichoic acid of foodborne bacteria to cause membrane perturbations (Raafat et al. 2008; Lee and Je 2013). In contrast, the capsular polysaccharide layer, lipopolysaccharides, and outer membrane proteins of Gram-negative pathogens may act as barrier for macromolecules and hydrophobic materials (ref). However,

at this stage the exact mechanism of action of CPCs against PA and LM is unknown and hence further detailed investigation on the possible cellular targets for CPCs against LM and PA needs to be investigated.

Biofilm inhibitory potential of CPCs

The in vitro anti-biofilm activity of CPCs was determined against LM and PA. In biofilm inhibition studies, UMC exhibited mean BIC (4.5) which was 4.1 folds higher than its MIC against PA. Similarly, the mean BIC of UMC (4.08) was 34-fold higher than its MIC against LM (Table 1 and S1). However, the mean BIC value of CFA, CCA, and CSA (0.61, 0.68, and 0.83 mg/ml, respectively) was 5.4–7.3-fold lower than BIC of UMC against PA. Similarly the mean BIC values of CFA, CCA, and CSA (0.85, 0.42, 0.72 mg/ml for, respectively) was 4.8-, 9.71-, 5.6-fold lower than BIC of UMC against LM strains. The BIC values based on OD determination were also consistent with the biofilm biomass estimated by safranin method (Data not shown). Earlier Tan et al. (2011) showed that carboxymethyl chitosan exhibited BIC values of 1.5–2 mg/ml (> 67% of inhibition) against SA and PA biofilms. However, our BIC values are comparable with the values reported for chitosan-propolis (Panwar et al. 2016) against *Enterococcus faecalis* (BIC, 0.3 mg/ml, > 90% inhibition).

The BEC of UMC against PA and LM (17.4, 23 mg/ml, respectively) was 9.4, 135 folds higher than their respective MICs, indicating that UMC was inactive against mature biofilms. The effect of specific MW of chitosan against PA and LM biofilms was earlier reported (Mu et al. 2014). Interestingly CPCs exhibited BEC values (3.35 mg/ml for CFA, CCA, and 3.70 mg/ml for CSA) that were 4.7–5.1 folds lower than UMC against PA and LM, suggesting that

Table 1 Minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), biofilm inhibitory concentration (BIC), and biofilm eradication concentration (BEC) determination for unmodified chitosan (UMC) and chitosan phenolic conjugates (CPCs) against *Pseudomonas aeruginosa* (PA) and *Listeria monocytogenes* (LM)

CPCs ^a	MIC (mg/ml)		MBC (mg/ml)		BIC (mg/ml)		BEC (mg/ml)	
	Range	<i>G</i> -mean ^b	Range	<i>G</i> -mean ^b	Range	<i>G</i> -mean	Range	<i>G</i> -mean
PA (<i>n</i> = 7)								
UMC	0.40–2.04	1.08	1.02–4.0	1.84	1.02–16.3	4.50	3.27–65.5	17.4
CFA	0.12–0.51	0.22	0.12–1.02	0.30	0.25–2.04	0.61	2.04–8.19	3.35
CCA	0.12–0.51	0.24	0.25–1.02	0.37	0.25–2.04	0.68	2.04–8.19	3.35
CSA	0.12–1.02	0.33	0.12–1.02	0.45	0.25–16.3	0.83	2.04–8.19	3.70
LM (<i>n</i> = 4)								
UMC	0.06–0.51	0.12	0.12–0.51	0.17	2.04–16.3	4.08	16.3–32.7	23.0
CFA	0.03–0.12	0.05	0.03–0.12	0.06	0.51–2.04	0.85	1.02–2.04	1.71
CCA	0.03–0.12	0.05	0.03–0.25	0.08	0.12–1.02	0.42	1.02–2.04	1.71
CSA	0.06–0.12	0.08	0.06–0.25	0.12	0.51–1.02	0.72	1.02–2.04	1.71

^aCPCs; chitosan-phenolic conjugates (UMC; unmodified chitosan, CFA; chitosan ferulic acid, CCA; chitosan-cafeic acid, CSA; chitosan-sinapic acid)

^bGeometric mean for MIC, MBC, BIC, and BEC are transformed data derived using logarithms to generate a normal distribution. Values in bold interface are statistically significant ($p < 0.01$)

phenolic grafting improved the antibiofilm activity of chitosan. The antibiofilm activity of Chitosan hydrides with alginate (0.04 mg/ml), iron oxide (0.5 mg/ml), streptomycin (0.5 mg/ml), and undec-10-enoic acid (5 mg/ml), and inulin (1 mg/ml) against various food pathogens including PA and LM has been reported (Thaya et al. 2018; Zhang et al. 2013, 2018; Laskar et al. 2017). Individual phenolics like ferulic and gallic acids were reported to inhibit mature biofilms of PA and LM (Borges et al. 2012). Conjugation of ferulic acid and propolis with chitosan was reported to inhibit mature biofilms of *Candida albicans* and *E. faecalis* (Panwar et al. 2016). In addition, here we reported about the biofilm eradicating role of CPCs against mature biofilms of PA and LM.

The effect of CPCs on the biofilm architecture of PA and LM was visualized using fluorescence microscopy (Fig. 1). Especially, biofilm treated with CPCs at $0.5 \times \text{BEC}$ exhibited altered biofilm architecture consisting of scattered micro colonies and cells with enhanced PI uptake suggesting that CPCs permeabilized PA and LM cells embedded

in the biofilm matrix. On the other hand, untreated controls exhibited homogenous biofilm architecture with no apparent PI uptake. Earlier chitosan was shown to interact with cell membrane, alters zeta potential and adhesion properties of *Candida albicans* in biofilms (Martinez et al. 2010). In this regard our PI uptake assays demonstrates that CPCs has profound impact on the membrane integrity properties of the biofilm cells (PA and LM), which may translate into interference with adhesion, cell–cell interactions in mature biofilms. However, more experimental work is needed to support such a claim.

CPCs prevent biofilm formation

Sub MIC of CPCs dose dependently inhibited biofilm formation by PA (KCTC 11321) and LM (KCTC 3569). Particularly, CFA ($0.5 \times \text{MIC}$) significantly ($p < 0.001$) reduced biofilm formation by 67.8 ± 0.8 , $79.3 \pm 0.9\%$ in case of PA and LM, respectively (Fig. 2a, b). Under the continuous exposure

Fig. 1 Chitosan phenolic conjugates increase membrane permeability of certain biofilm bacteria. *Listeria monocytogenes* KCTC 3569 (LM) or *Pseudomonas aeruginosa* KCCM 11321 (PA) biofilms were exposed to, un-modified chitosan (UMC), chitosan ferulic conjugate (CFA), –caffeic acid (CCA), and –sinapic acid (CSA). Cells treated with 1% acetic acid was used as control. (Scale: 10 μm for LM, 20 μm

for PA). The propidium iodide (PI) uptake by LM (a) and PA (b) in presence of CPCs as measured by fluorescence intensity ratio of PI to DAPI using confocal laser scanning microscope. Data are mean values ($\pm \text{SD}$) of two independent experiments with triplicates ($n=6$). p values less than 0.05 is considered statistically significant (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$)

of CFA, PA and LM exhibited reduced ability to form biofilms as evident from light microscopic observation of crystal violet stained biofilms on polystyrene plates (Fig. 2c). For most of other strains, presence of $0.5 \times \text{MIC}$ of UMC, CCA, CFA, and CSA the mean biofilm values were equal to 52.8, 47.9, 44.5, and 43.5% for PA, and 61.4, 56.7, 50.3, and 48.6% for LM, respectively (Table S2). Tan et al. (2011) reported that carboxymethyl chitosan (2.5 and 0.15 mg/ml) reduced SA and PA biofilms by 74.6 and 81.6%, respectively. Our results are in line with the anti-biofilm potential of phenolics such as propolis, ferulic acid, proanthocyanidin functionalized chitosan against various pathogens (Ong et al. 2017; Panwar et al. 2016; Song 2013).

The biofilm preventive role of CPCs is not clear at this stage hence it is our further interest to study the interaction

between poly cationic CPCs with the bacterial cell membranes and their effect on surface colonization, adhesion, and cell–cell interactions during biofilm formation by PM and LA. Further, inhibition of expression of *LasI*, *LasR*, *RhlI*, and *RhlR* genes in PA and *flaA*, *fliP*, *fliG*, *flgE*, *motA*, *motB*, *agrA*, *agrB* and *agrC*, *dnaK*, *prfA*, *degU*, and *mogR* in LM are implicated in the inhibition of adhesion, biofilm formation and quorum sensing of PA and LM, respectively (Karatan and Watnick 2009). Hence a more detailed study on the effect of CPCs on the expression of biofilm related genes in PA and LM is also further warranted.

Fig. 2 Inhibition of biofilm formation of *Pseudomonas aeruginosa* (a) and *Listeria monocytogenes* (b) by chitosan phenolic conjugates (CPCs). Biofilms were grown on polystyrene surface in the presence of sub inhibitory concentration of CPCs and biofilm formation value was represented in percentage of control. Data are mean values ($\pm$ SD) of two independent experiments with triplicates ($n=6$). p values less than 0.05 is considered statistically significant (* $p<0.05$; ** $p<0.01$; *** $p<0.001$). Microscopic visualization of biofilm for-

mation by LM (top row) and PA (bottom row) in presence of sub MIC of CFA on polystyrene surface (c). Biofilms (5×10^5 cfu/ml) were grown over night on polystyrene plates (1×1 cm) immersed in 24 well microtiter plates containing TSB (1% glucose) previously adjusted with sub-lethal doses of CPCs. After 24 h of incubation, the plates were washed twice with PBS and fixed with glutaraldehyde (4%) and stained with crystal violet (0.4%) as described by Xu et al. (2016) before light microscopic observation. (Scale; 20 μ m)

Conclusion

In continuation to our previous work on the antimicrobial efficacy of CPCs against methicillin-resistant *Staphylococcus aureus* (Eom et al. 2016) we here show that CPCs exhibits better antimicrobial and anti-biofilm activity against foodborne pathogens with the ability to interfere with bacterial adhesion. Data in the literature on the acute and long term effects in vivo suggested that chitosan and its derivatives are safe without significant toxicity. Further due to biocompatibility, CPCs may be suitable antimicrobial agents for applications in food systems to prevent biofilm formation and spread of foodborne pathogens. A further thorough investigation on the antimicrobial and anti-biofilm mechanism of action of CPCs is thus further warranted.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest regarding the publication of this manuscript.

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